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DNA methylation and carcinogenesis in digestive

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Reviews

DNA methylation and carcinogenesis in digestive neoplasms

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The DNA of vertebrates contains tissue specific patterns of methylated cytosine residues. These methylation patterns are transmitted by clonal inheritance[1] through the strong preferance of mammalian DNA (cytosine-5) methyltransferase (DNA-MTase) for hemimethylated DNA[2], and are established during early embryogenesis and gametogenesis[3]. Newly replicated DNA lacks this methylation in the nascent strand. Shortly after the passage of the replication fork, a maintenance DNA-MTase methylates CpG dinucleotides on the newly synthesized strand, thereby recreating the spectrum of methyl groups that existed prior to replication. DNA methylation (5-methylcytosine[5-mCyt] content of DNA) plays a considerable role in both normal development and carcinogenesis[4], but there seems to be conflicting reports concerning the role of DNA methylation in carcinogenesis. It has been hypothesized that hypomethylation of DNA facilitates aberrant gene expression in tumorigenesis[5]. Some believe that hypermethylation of DNA leads to the causative alteration in tumorigenesis which involves inactivating tumor suppressor genes and marking chromosome regions for deletion[6]. Some overlooked the importance of alterations in gene expression and thought that mutation played the key role[4]. All these help to achieve a better understanding of the mechanisms underlying carcinogenesis. We believe that carcinogenesis is a multistep/multistage process that occurs in animals[7] with more carcinogenesis than mutagenesis, and that DNA methylation played multiple roles in the transformation from a normal cell into a frank malignancy.

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DNA METHYLATION AND CANCER

Mutations which occur at CpG dinucleotides in vertebrate DNA can be attributed to the hydrolytic deamination of 5-mCyt and are easily recognized because of the nature of base substitution. Deamination of 5-mCyt dinucleotides results in the formation of thyminephosphoguanosine (TpG). This still does not account for the high frequency of mutagenesis observed at CpG sites. Differences in the repairing efficiencies of promutagenic lesions may be partly responsible for this discrepancy. The guanosine-thymine (G-T) misrepairs resulting from deamination of 5-mCyt are believed to be more difficult for the cell to repair, as thymine is a normal component of DNA.

Mutations in the evolutionarily conserved codons of the p53 tumor suppressor gene are common in diverse types of human cancer. The p53 mutational spectrum differs among cancers of the colon, esophagus and liver. Transitions predominate in colon, whereas G:C to T:A transversions are the most frequent substitutions observed in cancers of the liver. Mutations at adeninethymine (A:T) base pairs are seen more frequently in esophageal carcinomas than in other solid tumors. Most transitions in colorectal carcinomas are at CpG dinucleotide mutational hot spots. G-T transversions in esophageal carcinomas are dispersed among numerous codons[8]. One copy of the short arm of chromosome 17, which harbors the p53 gene, is lost in many human tumors including those of the colon and rectum. In the colon carcinomas 75% - 80% show a loss of both p53 alleles, one through deletion, the other through a point mutation. The point mutations are usually misense, giving rise to an altered protein[9]. Cytosine-thymine (C-T) transitions at CpG sites are the most prevalent mutations found in the p53 tumor suppressor gene in human colon tumors and in the germline (Li-Fraumeni syndrome). All of the mutational hot spots are methylated to 5-mCyt, and it has been hypothesized that the majority of these mutations are caused by spontaneous hydrolytic deamination of this base to thymine[10]. It is estimated that 75%-90% of hepatocellular carcinoma (HCC) cases are attributable to hepatitis B virus. HBV DNA integrates into HCC cells at random sites in the genome. It contains the X gene, which codes for a protein HBX that modu-

lates the transactivation of many cellular genes and is a candidate viral oncoprotein. HBX protein binds with p53 in vitro and in vivo[11], inhibits p53 sequence-specific DNA binding and transactivation activities, partially disrupts p53 oligomerization and prevents p53 binding to transcription-repair coupling factor ERCC3[12]. HBV infection alone does not influence the rate of p53 mutation, and aflatoxin B1 (AFB1) exposure is the most important factor influencing mutation prevalence. AFB1 plays an etiological role in HCC carcinogenesis and indicates a synergy between HBV and AFB1[13]. In patients with liver tumors from geographical areas where both AFB1 and HBV are cancer risk factors, most mutations are at one nucleotide pair of codon 249[8]. Multiple gene-tic alterations occur in gastric carcinomas, including point mutation of the *ras* oncogene and the p53 tumor suppressor gene, amplification of the c-met, k-sam, and c-erbB-2/neu genes, and loss of heterozygosity (LOH) of the bcl-2, APC, and DCC genes. Genetic changes in the transforming growth factor beta (TGF-β) type II receptor gene or altered expression of its messenger RNA[14] occur commonly in gastric cancer cells resistant to growth inhibitory action of TGF-β. Inactivation of the p53 gene through mutations and the allelic deletion may play an important role in gastric tumorigenesis. These mutations may cause a conformational change in the p53 protein, resulting in the loss of p53 suppression of the gastric cell growth partly through disruption of the association of p53 protein with a cellular component[15]. Allelic loss and mutation of the p53 gene are detected in over 60% of gastric cancers regardless of the histological type. Several gastric cancer cell lines have shown p53 gene abnormalities[16]. The c-met gene encoding the receptor for hepatocyte growth factor (HGF) is often amplified in advanced gastric cancer, particularly in 39% of scirrhous carcinomas, whereas c-met amplification is extremely rare in esophageal and colorectal cancers[17]. The interaction of c-met overexpressed tumor cells and HGF from activated stromal cells is involved in the morphogenesis and progression of gastric cancer. The K-sam gene encoding receptor for keratinocyte growth factor is amplified preferentially in the poorly differentiated scirrhous cancer[18]. However, no K-sam amplification is seen in esophageal and colorectal cancers. In general, K-sam amplification is independent of c-met amplification in scirrhous carcinoma, and c-erbB-2 gene amplification is detected only in well differentiated gastric cancer. Moreover, overexpression of c-erbB-2 is closely correlated with livermetastases. c-Ki-ras point mutation is observed in 9%-18% of well differentiated gastric cancers but not in poorly differentiated ones[19].

MECHANISMS OF DNA METHYLATION IN CARCINOGENESIS

Both general hypomethylation and regional hypermethylation coexist in the genome of a wide variety of human and animal cancers[5,6]. It has been suggested that changes in methylation may not play a causal role in carcinogenesis and could be consequences of the transformed state of tumor cells and that C-T transitions brought about by increased expression of the DNA methyltransferase play the key role[4]. Beside DNA MTase induced mutations additional factors appear to be involved in the cancer process. Cancers originate from a single cell that is changed dramatically by a series of alterations to the genome, e.g., mutation and changes in methylation altered gene expression. Mutagenesis plays a role in carcinogenesis. Mutated gene must be expressed to exert an effect.

It has been suggested that reduction of DNA MTase activity would lead to marked hypomethylation which can inhibit tumorigenesis[4,20]. Since DNA methylation is critical in development and differentiation[21] of tumors, it is reasonable to propose that hypomethylation at an intermediate level plays a key role in carcinogenesis while excessive hypomethylation may not be compatible with the life of the affected cells (e.g., owing to massive deregulation of gene expression). By this we mean that initiated cells may die under the severe conditions of hypomethylation and would not be available to form tumors. Thus the fact that inhibition of methylation may decrease tumor formation does not prove that excessive DNA MTase activity is the sole mechanism underlying carcinogenesis.

There are mechanistically and theoretically plausible genotoxic mechanisms that support the roles of both hypomethylation and hypermethylation of DNA (i.e. epigenetic changes) in carcinogenesis. These involve mainly alterations in normal gene expression (including tumor suppressor genes). Altered DNA methylation not only affects gene expression but also facilitates mutations, as 5-mCyt can deaminate spontaneously to Thymine (T). This indicates that elevated expression of the DNA MTase may lead to increased 5-mCyt, and this can increase the probability of C to T transitions[4,22,23]. Moderate DNA-MTase increases are not merely bystander effects, but could rather constitute one of the earliest and fundamental changes of neoplastic development[23]. Hypomethylation of DNA is associated with increased gene expression[24]. Additionally, a decreased capacity or fidelity of maintaining the normal methylation status of DNA may underlie the sensitivity of some mouse strains to liver tumorigenesis, in which increased expression of oncogenes appears to be involved by facilitating tumor promotion[5,25,27]. The

principal characteristic of the promotion stage of the carcinogenesis that distinguishes it from the stages of initiation and progression is its operational reversibility, i.e., clones of initiated cells regress when the promoting agent is withdrawn. The promotion stage ends when a lesion attains the capacity for growth in the absence of a promotion stimulus, i.e., when it is no longer reversible and can progress to a frank malignancy[7]. Hypermethylation of specific regions of DNA has been identified in cancer cells. For example, the Von Hippel Landau (VHL) tumor suppressor gene is hypermethylated and inactivated in a fraction of renal cell lines and tumors that did not have mutations in the coding regions sequenced[6]. Regional chromosomal hypermethylation is related to areas believed to contain tumor suppressor genes at various target sites. This regional hypermethylation in portions of the genome normally unmethylated may inactivate tumor suppressor genes. The functional significance would be the same as an inactivating mutation or as the loss of an allele. Regional hypermethylation of the retinoblastoma gene appears to inhibit transcription of this tumor suppressor genes[26]. Hypermethylation of the 5'CpG island of CDKN2/p16/MTS (tumor suppressor gene located on chromosome 9p21) is frequent in cell lines and primary lung tumors, gliomas and head and neck squamous cell carcinomas. Furthermore, inactivation through DNA methylation can occur not only in neoplasms where deletion is frequent in cell lines (breast, renal cells) but also in those which are not commonly associated with loss of p16 through homozygous deletion (colon and prostate). Hypermethylation of the p16 gene promoter region is a common abnormality of p16 gene in human cancer[27]. Nearly all acute leukemias and 50% of chronic leukemias and lymphomas show extensive methylation of the estrogen receptor (ER) gene CpG island[28]. Mutation in the p53 tumor suppressor gene is believed to occur in more than half of all solid tumors[29]. Approximately 24% of point mutations of p53 in human cancers are C-T transitions at CpG dinucleotides[29]. All of the mutational hot spots in the p53 gene occurring at CpG are methylated[30-32], suggesting an involvement of 5-mCyt as an endogenous mutagen. It is important to not only determine which tumor suppressor genes lie in specific regions and their normal functions but also demonstrate a causative role, perhaps by reversing their inactivation. Altered DNA methylation leads to 5-mCyt which has intrinsic hypermutability as compared with C via deamination. This causes C-T transitions[22]. In the presence of low levels of S-adenosyl methionine, DNA-MTase may be able to form uracil leading to C-T transitions[22]. Therefore, the high rate of mutation at CpG dinucleotides may be due, in part, to DNA-MTase mediated deamination[4,23], and inhibition of DNA mismatch repair£Û23£Ý. Additionally, 5-mCyt may influence carcinogenesis via inhibition of DNA repair, leading to fixation of promutagenic lesions[5,25]. However, the high percentage of mutations at CpG sites can not be explained solely by the presence of 5-mCyt, as the methylation patterns of the p53 gene are tissue independent, suggesting that tissue specific methylation does not contribute to the different mutation patterns at CpG sites seen in tumors[32]. This supports the contention that the effect of DNA methylation alterations may vary in different tissues and in the genesis of different tumor types.

The complex nature of the methylation role in region of gene expression is illustrated by the insulin growth factor type II receptor (Igf2r) gene. Both hypomethylation of the 5' flanking region and methylation of a specific CpG site in an intron are required for the occurrence of expression. The latter appears to be an imprinting signal, and hypomethylation at this site may silence the gene even if the 5'flanking region remains hypomethylated[33].

The epigenetic properties of DNA methylation are heritable and unlike the mutagenic effects of 5-mCyt which do not involve alterations of the primary DNA sequence. Methylation of cytosine residues contained in CpG islands of certain genes has been inversely correlated with gene activity, but it is still unclear whether this methylation is actually responsible for different activity states of a gene or is merely the result of such changes. Methylation at a CpG islands may lead to decreased gene expression by a variety of mechanisms including disruption of local chromatin structure, inhibition of transcription factor DNA, or by recruitment of proteins which interact specifically with methylated sequences indirectly preventing transcription factor binding[5,34]. Increased methylation and heterochromatization of CpG islands have been proposed as a mechanism for silencing the expression of non-essential genes during the establishment of immortal cell lines.

CONCLUSION

The current literature provides a compelling basis for suggesting that mutations arising secondarily to deamination of 5-mCyt, C or both are an important source of critical point mutations. Mutation, altered gene expression, hypomethylation and hypermethylation may be all related to carcinogenesis which are not mutually exclusive. Hypomethylation, hypermethylation and mutations may be important based on different situations. The examination of DNA methylation status provides the potential to discover alterations in gene expression, cell proliferation, mutation, chromatin aberrations and inactivation/

deletion of tumor suppressor genes in multifaceted approach that fits the multistep process of carcinogenesis. This notion can be supported by the depiction of human colon carcinogenesis in which roles for hypomethylation of DNA, mutation and tumor suppressor gene inactivation are considered to be relevant to the ultimate tumor formation[5,25]. There is a need to address the functional significance of specific changes in methylation (e.g., how the binding of transcating factors to specific genes is affected by methylation), and changes in methylation that occur in target tissues prior to the appearance of frank malignancies. The overall goal should be an understanding of changes in methylation and how they facilitate movement of cells through the different stages of carcinogenesis. This can be accomplished by keeping in perspective the fact that cancer is a disease of the whole entity, and thus there is a need to focus, though not exclusively, on in vivo studies.

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